[SEQ CHAPTER \h \r 1]**Primary Reviewer:** Cassandra Kirk, Ph.D., Biologist, Emerging Technologies Branch **Date:** 2/26/20

[SEQ CHAPTER \h \r 1]

[SEQ CHAPTER \h \r 1]Secondary Reviewer: Chris Wozniak, Ph.D., Biotechnology Special Assistant, OPP/BPPD **Date:**

DATA EVALUATION RECORD

[SEQ CHAPTER \h \r 1]**REQUIREMENT:** EPA OCSPP

TEST MATERIAL (PURITY): *Aedes aegypti* OX5034

SYNONYMS: OX5034

CITATION: Tetracycline-Repressible Transactivator Protein Variant (tTAV - OX5034 OX5034) and Related Genetic Material from OX5034 *Aedes aegypti*: Request for Waiver from the Wild Mammal Toxicity Testing. Volume 23, EUP Submission; MRID 50889422, July 16, 2019

SPONSOR: Oxitec Ltd, 71, Milton Park, Abingdon, Oxfordshire, OX14 4RX United Kingdom

AUTHOR: Oxitec Ltd.

TEST SITE: Not applicable

COMPLIANCE: This document is a waiver request from the requirement to conduct a wild mammal toxicity study (OCSPP 850.2400) for OX5034 *Aedes aegypti*. As such, Good Laboratory Practice Standards, 40 CFR Part 160, are not applicable.
This DER does not contain FIFRA CBI.

EXECUTIVE SUMMARY:

The developer of the male-sterile *Aedes aegypti* OX5034 mosquito, Oxitec Ltd., requests a waiver for performing a wild mammal toxicity study through application of rationale outlining the reasons that such a test is unnecessary. Oxitec has indicated that wild mammal exposure to the DsRed2-OX5034 and tTAV-OX5034 proteins via an oral route is likely to be rare and that if wild mammals inadvertently ingested OX5034 male mosquitoes, the risk to wild mammals would be negligible as there is no potential health hazard because tTAV-OX5034 and DsRed2-OX5034 are expressed at negligible *de minimis* quantities in OX5034 male mosquitoes.

Although OX5034 has not been tested on any wild mammal species, feeding studies on fish and invertebrates have shown no evidence of any adverse impacts. For example, a study submitted in support of this experimental use permit demonstrated a lack of toxicity or adverse effects to guppies (*Poecilia reticulata*) fed OX5034 larvae (MRID 50698708). American signal crayfish (*Pacifastacus leniusculus)* similarly consumed OX5034 larvae without any evidence of adverse effects (MRID 50698707).

Based upon bioinformatic analyses, neither DsRed2-OX5034 or tTAV-OX5034 are known to share significant sequence homology with known toxins (MRID 50889420). The tTAV-OX5034 protein does not share significant homology with any known or putative allergens, however the Agency has not made a determination regarding the allergenicity of DsRed2. Both of these proteins are predicted as susceptible to several proteases found in the human gastric system (*i.e.* pepsin, trypsin, chymotrypsin) based upon bioinformatics analysis (MRID 50889420), thus proteins are expected to be broken down following ingestion. Based upon bioinformatics analyses, both DsRed2-OX5034 and tTAV-OX5034 are also predicted as susceptible to two environmental proteases (*i.e.* proteinase K and subtilisin A) and are thus expected to degrade under field conditions. While several variants of DsRed can sometimes exhibit toxic effects within living cells, oral consumption and subsequent digestion would result protein degradation, thus uptake of the intact protein into cells following ingestion is unlikely. Because biting females will not be released, wild mammals will not serve as bloodmeals for mosquitoes carrying tTAV-OX5034 and DsRed2-OX5034 proteins, thus excluding this as an exposure pathway to these proteins.

PURPOSE OF THE STUDY:

The OCSPP guideline 850.2400 is intended for use in developing data, specifically either acute toxicity data, such as the acute oral median lethal dose (LD_{50}) or dietary median lethal concentration (LC_{50}), or longer term continuous or repeated exposure no-observed-effect level (NOEL) or no-observed-effect concentration (NOEC) data from subchronic studies for wild mammals of chemical substances and mixtures ("test chemicals" or "test substances") subject to environmental effects test regulations. While the LD_{50} or LC_{50} studies are specifically designed to allow calculation of the LD_{50}/LC_{50}, the study can be used to obtain information regarding sublethal effects which are used in Agency evaluations. The Environmental Protection Agency uses these and other data to assess the hazard and risks to wild mammals that these chemicals may present through environmental exposure.

CLASSIFICATION: ACCEPTABLE

I. MATERIALS AND METHODS:

A. GUIDELINE FOLLOWED: OCSPP Guideline 850.2400

Deviations from guideline: Waiver request.

B. MATERIALS:

1. **Test Material:** *Ae. aegypti* OX513A-tTAV / DsRed2-OX5034

   Control Substance: N/A

2. **Test Organism:**

   Species (common and scientific names): N/A

   Age at study initiation: N/A

   Number of test individuals /Sex: N/A

Strain/Source: OX5034 was developed via standard micro-injection methods (Morris, 1997; Jasinskiene et al., 1998),by injecting a combination of pOX5034 plasmid DNA (containing the tTAV-OX5034 and DsRed2-OX5034 genetic material) and *piggyBac* mRNA as the source of transposase, into *Aedes aegypti* mosquito eggs of an arbovirus free Latin American wild-type strain (originating from Chiapas, Mexico, and held in Oxitec labs since 2006). The transposase mRNA provides a source of *piggyBac* transposase, to allow the rDNA construct to be integrated into the germline of *Aedes aegypti*. The non-autonomous transposon has no endogenous source of transposase in mosquitoes and has had no further translocation. The resulting OX513A line has been maintained in a continuously cycling insect colony for the equivalent of over 27 generation equivalents. Sterile males, homozygous for the two transgenes, are to be released for population suppression; a very low number of homozygous (tTAV-OX5034/ DsRed2-OX5034). When male OX5034 *Aedes aegypti* homozygous for the conditional female-specific self-limiting gene (carrying two copies of the gene) are released into the environment and mate with wild *Aedes aegypti* females, their offspring inherit a single copy (so are hemizygous) of the self-limiting gene. The self-limiting gene kills only female offspring (carrying one copy of the self-limiting gene), which die at early larval stages of development, while hemizygous males will survive to pass the OX5034 genes on further. Hence the OX5034 mosquito can be considered to be a species-specific female larvicide for *Aedes aegypti*.

BPPD Comments:

Based upon bioinformatic analyses, neither DsRed2-OX5034 or tTAV-OX5034 are known to share significant sequence homology with known toxins (MRID 50889420). The tTAV-OX5034 protein does not share significant homology with any known or putative allergens, however the Agency has not made a determination regarding the allergenicity of DsRed2. Both of these proteins are predicted as susceptible to several proteases found in the human gastric system (*i.e.* pepsin, trypsin, chymotrypsin) based upon bioinformatics analysis (MRID 50889420), thus proteins are expected to be broken down following ingestion. Based upon bioinformatics analyses, both DsRed2-OX5034 and tTAV-OX5034 are also predicted as susceptible to two environmental proteases (*i.e.* proteinase K and subtilisin A) and are thus expected to degrade under field conditions. While several variants of DsRed can sometimes exhibit toxic effects within living cells, oral consumption and subsequent digestion would result protein degradation,

thus uptake of the intact protein into cells following ingestion is unlikely. Because biting females will not be released, wild mammals will not serve as bloodmeals for mosquitoes carrying tTAV-OX5034 and DsRed2-OX5034 proteins, thus excluding this as an exposure pathway to these proteins.

Aedes aegypti is known to frequent households and associated habitat in close proximity to buildings inhabited by humans, thus limiting exposure for wild mammal not associated with human habitation. This is in part a consequence of their preference for human blood as a source of nutrients to supply developing eggs (Scott *et al.,* 2000; Harrington et al., 2001; Ponlawat and Harrington, 2005) and utilization of containers for oviposition and larval development. *Ae. aegypti* do, however, utilize other mammalian and avian sources for bloodmeals, including dogs, cats, cattle, horses, swine and chickens (Jansen *et al.,* 2009; Barrera *et al.*, 2012). Local climatic conditions and geography (*e.g.,* degree of urbanization) may affect the distribution of *Ae. aegypti* in a particular season (Scott *et al.*, 2000). There are domestic, sylvan and peridomestic forms of *Ae. aegypti* which predominately reside in urban / household, forested, or environmentally modified areas (*e.g.,* coconut groves, farms), respectively; however, not all of these forms may exist in any one area or country (Tabachnik *et al.*, 1978).

Mosquitoes tend to make up a small portion of the diet of most insectivorous mammals. Chiropteran species are considered active generalist predators of insects and it has been anecdotally suggested that insectivorous bats may consume 1000 or more mosquitoes per hour or approximately 12,000 per night. This suggestion stems in part from extrapolations of a study (Griffin *et al.*, 1960) conducted in a sealed environment wherein mosquitoes were the only prey made available to captive bats. The intent of the study was to evaluate echolocation characteristics of *Myotis* spp. in finding *Culex quinquefasciatus*, the southern house mosquito, on the wing, not to establish the bat's dietary preferences. In areas where larger, more nutritious insect prey are available, bats do not consume large numbers of mosquitoes as they do not constitute significant calories or nutrients relative to the task of predating upon them (Gonsalves *et al.,* 2013; Wetzler and Boyles, 2018). While northern bats (*Myotis septentrionalis*) readily consume mosquitoes in enclosures, evidence suggests that they consume few mosquitoes in an open feeding environment (Boyles et al., 2013).

A study of Big Brown Bats' (*Eptesicus fuscus*) prey preferences, a generalist feeder, indicated a predominance of Coleoptera, Diptera, Ephemeroptera and Lepidoptera species in their diet with Dipteran species predominated by chironomids and very few mosquitoes (Clare *et al.,* 2014). Similarly, a comparison of the diet of eight bat species in southern Illinois concluded that mosquitoes represented a small portion of the overall diet of these insectivorous bats (Feldhamer *et al.,* 2009).

Under certain conditions, such as colder nights where larger insects were less available or when female bats are lactating, Diptera, including mosquitoes and crane flies, may constitute a larger portion of the diet of the southeastern brown bat, *Myotis austroriparius*, in Florida (Zinn and Humphrey, 1983). These Dipterans constituted as much as 75% of biomass sampled by bats on cooler nights, however, the diversity of the diet of this insectivorous bat increased considerably during warmer temperatures (*i.e.*, most spring and summer nights). In a recent Wisconsin study, little brown bats (*Myotis lucifugus*) and big brown bats (*Eptesicus fuscus*) were found to include

mosquitoes (9 species identified) in their diet at 72% and 33% of samples, respectively, at all sites sampled (Wray *et al.,* 2018). In contrast, Whitaker and Lawhead (1992) found mosquitoes in 17% of fecal samples of *M. lucifugus* which constituted 1.8% (volumetrically) of their insect-based diet. Given the taxonomic, temporal and geographical breadth of the studies referenced above, it can be concluded that for different insectivorous bat species, mosquitoes may constitute less or more of their overall dietary intake of insects depending in part on seasonality, bat species and availability of diverse prey.

The OX5034 *Ae. aegytpi* releases will contain 100% male mosquitoes and therefore will not constitute a hazard related to female mosquitoes biting animals or transmitting disease, such as those caused by arboviruses. The OX5034 laboratory colony is evaluated for the presence of several different arboviruses and lots are rejected if the presence of any of these was confirmed.

As noted above, *Ae. aegypti* is predominantly a peridomestic resident and focuses primarily on human hosts when seeking a bloodmeal. The urban nature of this species and its preference for humans as a source of a bloodmeal make interactions with wild mammal species far less likely than with many other mosquito species.

The waiver request is appropriate considering the minimal exposure of wild mammal species to released OX5034 mosquitoes.

CONCLUSION: ACCEPTABLE

REFERENCES:

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